Attorney Docket No: 10969/2012 (Serial No.:09/821,654)

Hosoya et al.

Filed: March 29, 2001

Amendment After Final Office Action

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AMENDMENTS

In the Claims

Please amend claims 1, 3, 5-7, 9-11 and 13-14 as follows:

1. (Twice Amended) A conditionally immortalized cell established from a transgenic animal into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced, and wherein the cell exhibits an inside-outside polarity when cultured in vitro, and is capable of taking up a drug, and wherein the cell is immortal at conditions below 39°C, and wherein the cell does not contain a heterologous antibiotic resistance gene.

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3. (Twice Amended) An established cell derived from retinal capillary endothelial cells, which expresses a temperature sensitive SV40 large T-antigen gene, GLUT-1 transporter, and p-glyoprotein, and wherein the cell exhibits an inside-outside polarity when cultured in vitro, and is capable of taking up a drug, and wherein the cell is immortal at conditions below 39°C, and wherein the cell does not contain a heterologous antibiotic resistance gene.

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5. (Twice Amended) A method of establishing a conditionally immortalized cell which expresses a temperature sensitive SV40 large T-antigen gene, GLUT-1 transporter, and p-glycoprotein, and wherein the cell is immortal at conditions below 39°C, and wherein the cell does not contain a heterologous antibiotic resistance gene, the method comprising treating retinal capillary vessels of a transgenic animal into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced with protease and subculturing the resulting cells at 33°C.

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6. (Twice Amended) An established cell which expresses a temperature sensitive SV40 large T-antigen gene, GLUT-1 transporter, and p-glycoprotein, and wherein the cell exhibits an inside-outside polarity when cultured in vitro, and is capable of taking up a drug, and wherein the cell is immortal at conditions below 39°C, and wherein the cell does not contain a heterologous antibiotic resistance gene, the cell obtained by treating retinal capillary vessels of a transgenic animal into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced with protease and subculturing the resulting cells at 33°C.

- 7. (Twice Amended) An established cell derived from choroid plexus epithelial cells, wherein the cell exhibits an inside-outside polarity when cultured in vitro, and is capable of taking up a drug, which expresses a temperature sensitive SV40 large T-antigen gene, shows localization of Na⁺-K⁺ ATPase and GLUT-1 transporter in the cell membrane, and when cultured in a monolayer, shows the localization of Na⁺-K⁺ ATPase in the apical side, and wherein the cell is immortal at conditions below 39°C, and wherein the cell does not contain a heterologous antibiotic resistance gene.
- 9. (Twice Amended) A method of establishing a conditionally immortalized cell which expresses a temperature sensitive SV40 large T-antigen gene, shows localization of Na⁺-K⁺ ATPase and GLUT-1 transporter in the cell membrane, and when cultured in a monolayer, shows the localization of Na⁺-K⁺ ATPase in the apical side, and wherein the cell is immortal at conditions below 39°C, and wherein the cell does not contain a heterologous antibiotic resistance gene, the method comprising treating choroidal epithelium tissues of a transgenic animal into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced with protease and subculturing the resulting cells at 33°C.

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10. (Twice Amended) An established cell which expresses a temperature sensitive SV40 large T-antigen gene, wherein the cell exhibits an inside-outside polarity when cultured in vitro, and is capable of taking up a drug, and shows localization of Na⁺-K⁺ ATPase and GLUT-1 transporter in the cell membrane, and when cultured in a monolayer, shows the localization of Na⁺-K⁺ ATPase in the apical side, and wherein the cell is immortal at conditions below 39°C, and wherein the cell does not contain a heterologous antibiotic resistance gene, which is obtained by treating choroidal epithelium tissues of a transgenic animal into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced with protease and subculturing the resulting cells at 33°C.

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11. (Twice Amended) An established cell derived from brain capillary endothelial cells, wherein the cell exhibits an inside-outside polarity when cultured in vitro, and is capable of taking up a drug, which expresses a temperature sensitive SV40 large T-antigen, GLUT-1 transporter, p-glycoprotein, alkaline photosphatase, and γ- glutamyltransferase, and wherein the cell is immortal at conditions below 39°C, and wherein the cell does not contain a heterologous antibiotic resistance gene.

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13. (Twice Amended) A method of establishing a conditionally immortalized cell which expresses a temperature sensitive SV40 large T-antigen gene, GLUT-1 transporter, p-glycoprotein, alkaline phosphatase, and γ-glutamyltransferase, and wherein the cell is immortal at conditions below 39°C, and wherein the cell does not contain a heterologous antibiotic resistance gene, the method comprising treating brain capillary vessels of a transgenic animal into which a large T-antigen gene of SV40 temperature sensitive mutant tsA58 has been introduced with protease and subculturing the resulting cells at 33°C.